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Gyrodactylid Ectoparasites in a Population of Rainbow Trout (*Oncorhynchus mykiss*)

Rachel L Garcia,[1] Adam G Hansen,[2] Maia M Chan,[3] and George E Sanders[3,4,*]

A colony of rainbow trout (*Oncorhynchus mykiss*) in a decentralized aquatic animal facility was noted to have an increase in morbidity and mortality (from 4 or 5 fish each month to 3 or 4 fish daily) approximately 2 wk after experimental procedures began. The primary clinical signs were erratic swimming behavior and 'flashing' of fish against surfaces within housing enclosures. Moribund and normal rainbow trout were presented alive for diagnostic evaluation; samples of water from housing enclosures were provided for water quality assessment. The trout were determined to be infected with gyrodactylids, a common monogenean ectoparasite of the skin and gills in both marine and freshwater fish. This case report describes the diagnosis, pathology, and treatment of gyrodactylids and husbandry modifications associated with the resolution of this clinical aquatic-animal case.

Abbreviation: BBC, Big Beef Creek.

Here we describe a clinical case in which a population of hatchery-raised juvenile rainbow trout became infected with gyrodactylid ectoparasites after periodic exposure to wild-caught adult cutthroat trout, the most likely source of this foreign pathogen in the research facility. This parasitic infection ultimately caused a rapid and marked increase in morbidity and mortality above baseline levels. We discuss the acclimation and experimental procedures unique to the study design that led to the infection, its diagnosis, and its remediation in a research facility in which wastewater effluent could not be completely contained and neutralized. This report offers a unique example of pathogen transmission between groups of experimental animals and provides useful information for other clinical cases in which direct exposure of wild-caught and hatchery-raised fish is necessary for achieving research objectives or commercial endeavors.

Case Report

Animals and husbandry. Experiments were conducted at Big Beef Creek Field Station (BBC; Seabeck, WA), a remote decentralized facility of the University of Washington's School of Aquatic and Fishery Sciences. The facility consists of approximately 10 acres of natural and artificial rearing facilities, freshwater and dry labs, and indoor and outdoor rearing spaces. High-quality well water is the primary water source. Well depth is 300 ft, with the pump set at 220 ft below the surface. The only treatment the well water received before entering tanks containing fish involved in this case report was passage through packed column aerators for degassing and oxygenation. Historically, effluent from indoor rearing facilities passed through a preliminary catch basin, followed by UV disinfection, before entering a series of 3 larger settling ponds that ultimately emptied into the main stem of Big Beef Creek. However due to several issues, including the structural damage resulting from a 2007 flooding event, the UV disinfection system is nonfunctional, and wastewater entered the larger settling ponds directly during the study period.

Predatory-sized, wild cutthroat trout (*Oncorhynchus clarkii*; *n* = 28; fork length, 225 to 409 mm; Figure 1 A) captured from Big Beef Creek during August 2009 through January 2010 were held in 1 of 3 outdoor circular tanks (diameter, 4.1 m) supplied with 9.5 to 11.5 °C well water in a flow-through configuration at a rate of 10 to 12 gallons per minute (Figure 2 A). The fish were fed frozen krill (Hikari Bio-Pure , Hayward, CA) every other day. Newly captured cutthroat trout were visually inspected for external injuries, and only apparently healthy fish were retained (those that appeared unhealthy were returned to the creek immediately after capture). New cutthroat trout were not quarantined before introducing them into the existing population of cutthroat trout. Tanks used to house cutthroat trout were disinfected with a povidone–iodine solution (20 ppm; Argent Chemical Laboratories, Redmond, WA) prior to use.

Predatory-sized coho salmon (*Oncorhynchus kisutch*; fork length, 330 to 389 mm) had already been raised at and were available from BBC and were presumed to be SPF for external parasites. Coho salmon were held in the same type of tanks as the cutthroat trout but never at the same time. Tanks used to house coho salmon were disinfected with a povidone–iodine solution (20 ppm; Argent Chemical Laboratories) prior to use. The coho salmon were fed ad libitum rations of pelleted feed (Silver Cup Fish Feed, Salt Lake City, UT) while awaiting use in experimental trials.

Rainbow trout prey (*n* = 1520; fork length, 50 to 72 mm; Figure 1 B) were obtained in September 2009 from a certified disease-free hatchery (Eels Springs Hatchery, Washington Department of Fish and Wildlife, Shelton, WA). They were held indoors in 1 of 2 circular tanks (diameter, 1.5 m) receiving 9.5 to 11.5 °C well water in a flow-through configuration (Figure 2 B). Incandescent lights on a timer mimicked the natural photoperiod. Rainbow trout were fed various rations of pelleted feed (0.5% to 1.5% body weight every other day) to maintain required body sizes for experimentation. A holding tank designated as 'unused' contained rainbow trout that were naïve to predators (that is, had never encountered a predator) and awaiting use in a predation trial. A holding tank designated as 'used' held rainbow trout that

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[1]Department of Biological Structure, [2]School of Aquatic and Fishery Sciences, [3]Department of Comparative Medicine, University of Washington, and [4]Western Fisheries Research Center, Seattle, Washington.
[*]Corresponding author. Email: gsander@u.washington.edu

Figure 1. Typical (A) adult cutthroat trout and (B) juvenile rainbow trout used as predators and prey, respectively.

had originally been housed in the unused tank but which had been exposed to predators in the experimental arena during a predation trial. To acclimate them to predators, rainbow trout prey housed in either the experimental arena during pilot trials or in the unused holding tank were exposed once or twice each week to cutthroat trout predators, which were introduced into tanks beginning in October 2009.

The experimental arena was an indoor circular tank (diameter, 4.1 m; height, 1.2 m). The arena was lined with a fish-safe, flexible gray PVC material (shower pan liner from Oatey, Cleveland, OH). A curtain made from the same PVC material could be raised and lowered to split the arena into 2 halves and was used for acclimating predators and prey to the different light treatments for 1 h before initiating a trial. Light levels were controlled by 6 fluorescent fixtures wired to a series of dimming switches and were suspended above the surface of the water (Figure 3 A). After the light acclimation period, the curtain was lifted, and the predators were allowed to respond to the rainbow trout prey for 1 h, producing a total predator–prey exposure time of 2 h. The arena was shrouded with black plastic sheeting to remove external sources of light and other disturbances. A grid of black markers on the bottom of the arena was used to calibrate 2 overhead cameras for video analysis (Figure 3 B). Rainbow trout prey were either free-swimming or live-tethered to a weighted line, depending on the types of measurements required. Live tethering was accomplished by using monofilament fishing line to tie the connective tissue underneath the maxilla of the rainbow trout to a loop in the weighted line. This arrangement allowed the prey to ventilate, rotate freely around a central pivot point, and be pulled away easily when struck by a cutthroat trout.

After completion of a trial, the cutthroat trout were returned to an outdoor holding tank separate from other conspecifics awaiting use in a trial. Rainbow trout that sustained trauma (but were not killed) during a trial were fed to cutthroat trout that had already completed a trial. Rainbow trout that were uninjured after completion of a trial were placed into the used indoor holding tank. Species-dedicated nets and 5-gal buckets for cutthroat trout and rainbow trout were used to transport fish between holding tanks and the experimental arena. Nets and buckets were disinfected with a povidone–iodine solution (20 ppm; Argent Chemical Laboratories) after an entire set of experimental trials had been completed but were not disinfected between trials.

All research conducted by the investigative group using these fish species was approved by the IACUC of the University of Washington. The University of Washington is AAALAC-accredited.

Figure 2. Fish-holding tanks for (A) predatory cutthroat trout and coho salmon (outdoors) and (B) rainbow trout prey (indoors).

Case history, diagnostics, and treatment. The first signs of disease in both groups of rainbow trout were noted in mid- to late April 2010, 2 wk after experiments with cutthroat trout were initiated. During routine observations of the rainbow trout, the average mortality rate had increased to 3 or 4 fish daily from a previous average mortality rate of 4 or 5 fish per month (September 2009 through March 2010), for a cumulative mortality of 167 fish prior to treatment (80% of this mortality occurred in April 2010). In addition, researchers noticed several fish in both the unused and used holding tanks that were 'flashing' or rubbing against available interior tank surfaces. Given the rapid increase in morbidity and mortality, moribund and normal fish (n = 5 to 8 each) were recommended for evaluation via standard necropsy procedures. On the day of diagnostic evaluations, mortality had increased to 15 fish daily.

Moribund and normal rainbow trout (n = 10 each; fork length, 60 to 80 mm; weight, 3 to 6 g) were submitted live for necropsy. Fish were euthanized by using an overdose (250 mg/L) of tricaine methanesulfonate (MS222, Sigma–Aldrich, St Louis, MO) buffered to pH 7.5 with sodium bicarbonate (Church and Dwight, Princeton, NJ). Prior to euthanasia, several fish were swimming normally, but many were floating upside-down or sideways at the surface and were respiring slowly. The less-active fish had truncated pectoral, pelvic, and caudal fins. No other abnormalities were noted on gross examination of fish carcasses. Skin-scrape and gill biopsy samples were taken from 4 fish and examined microscopically via wet mount. Multiple

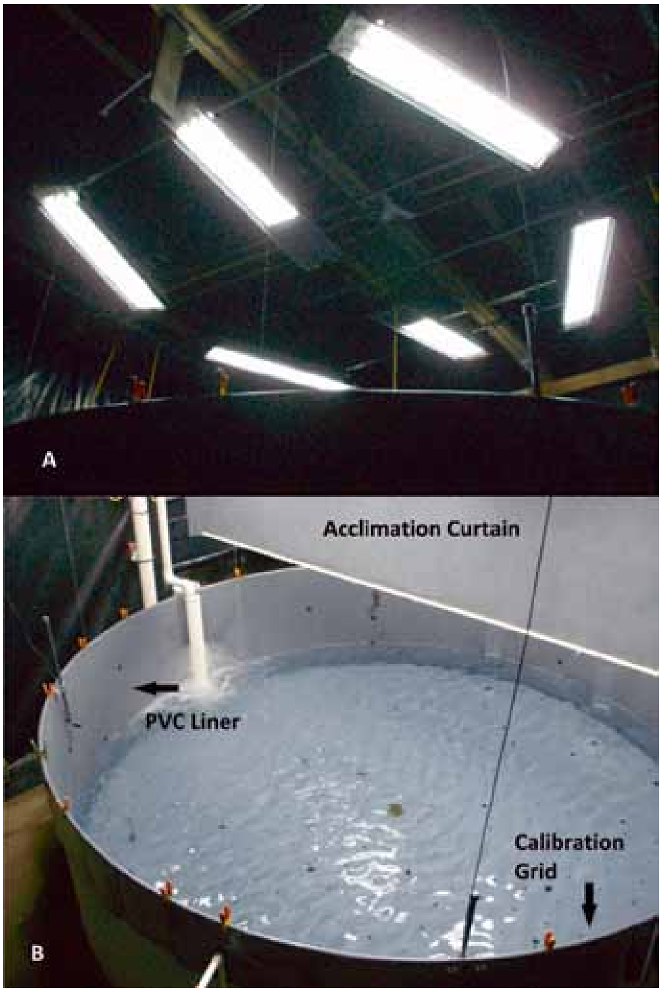

Figure 3. Experimental arena where rainbow trout prey were exposed to wild adult cutthroat trout during pilot and experimental trials. (A) Fluorescent lamps are suspended above the arena. (B) Inside the arena and its associated components.

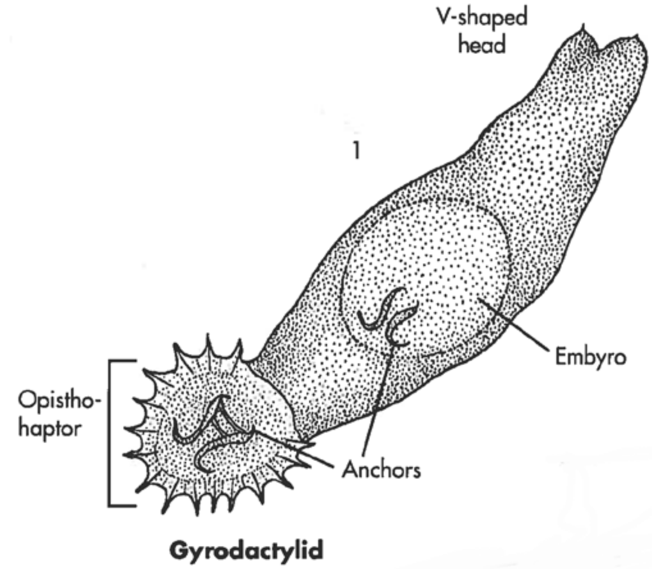

Figure 4. Gyrodactylid type monogenean. Note its size (0.3–1 mm), V-shaped head, lack of eyespots, developing embryo with anchors, and single pair of anchors. Modified from Fish Disease: Diagnosis and Treatment, Edward G Noga, 2010 Wiley Blackwell. Modified and reproduced with permission of John Wiley and Sons.

(15 to 20 per magnified field) monogeneans were identified on both the skin and the gills. The morphology of the opisthohaptor was characterized by the presence of single pair of centrally located anchors with multiple marginal hooklets.[5,18] In addition, the monogenean had a V-shaped head, lacked eyespots, and demonstrated a developing embryo with visible anchors.[5,18] According to similarities between the monogenean observed and a figure key (Figure 4), the monogeneans were classified as gyrodactylids.

Coelomic fluid and heart blood were sampled aseptically from 2 fish (one large, one small) and submitted for aerobic bacterial cultures and antibiotic sensitivities. Results from the bacterial cultures from the large fish revealed growth of coagulase-negative *Staphylococcus* spp. recovered from the broth subculture only. Cultures from the small fish grew *Enterococcus* spp. from the broth subculture only. These results were consistent with human-operator contamination during collection of the first sample and with fish gastrointestinal tract contamination during collection of the second sample; therefore, these organisms were not believed to be associated with the increased mortality rates. Testing of water-quality parameters included the following results: temperature, 11.2 °C; pH, 7.5; ammonia (NH_3), 0.0052 mg/L; alkalinity, 34.2 mg/L; total hardness, 85.5 mg/L; and conductivity, 158.7 µs. Nitrite (NO_2^-) was not tested because ammonia was very low. Evaluation and comparison with previous obtained values revealed that all tested water-quality parameters of the well-water source at BBC were within normal limits.

From these findings, we diagnosed ectoparasitism by monogenean flatworms as the primary cause of mortality. Several chemical therapies have been used to treat gyrodactylids,[18] but in this situation, treatment options were limited because effluents from holding tanks drained directly into Big Beef Creek watershed through 3 ponds (Figure 5). This situation precluded any possibility of effective containment of the effluent for chemical removal, leaving saltwater baths of affected fish as the only effective and environmentally acceptable therapy. Initial treatment plans specified that fish less than 5 g in weight were to be treated at 10 g/L for 10 to 15 min daily for at least 2 consecutive days; larger fish were to be treated at 15 g/L. To rule out potential adverse reactions to this treatment, sample groups of fish in both weight classes (test populations of 8 to 12 fish) were tested to see how they would respond. After experimentation with different salt concentrations, all size classes were found to respond well to 20 g/L of table salt (Morton Salt, Chicago, IL) for a 20-min contact time. Consequently, this modified treatment was applied once daily at the same time on 3 consecutive days to the remaining population of approximately 1300 rainbow trout. The water was aerated during treatment, and fish were monitored during and after treatment. After treatment, overtly sick fish were removed and euthanized. The greatest mortality occurred after the first bath, when a total of 44 fish representing both tanks died (or were euthanized). Only 23 fish in total died or were euthanized after the subsequent 2 bath treatments.

Husbandry changes recommended and implemented included cleaning of the challenge tank and all equipment and subsequent disinfection with povidone–iodine before and after each use. Because the coho salmon were destined for use as predators in the predation experiments and because they were of hatchery origin, they too would be susceptible to monogenean infection and would benefit from the added disinfection step between trials. Furthermore, an additional, dedicated 'return' tank was established for the rainbow trout that had been ex-

Figure 5. The last of the 3 settling ponds used to remove suspended solids in effluent from the Big Beef Creek hatchery facility before the water returns to Big Beef Creek.

posed to cutthroat trout, so that rainbow trout used in challenge experiments were no longer rehoused in the existing stock tanks. By 3 wk after treatment, total average mortality had returned to preexperimental levels (only 2 mortalities occurred from June through late August 2010). In addition, the coho salmon did not become infected with gyrodactylid monogeneans when used in successive trials as predators of rainbow trout.

Discussion

Monogeneans (parasitic flatworms) frequently infect the skin and gills of a wide range of marine and freshwater fish. These worms are classified into 2 groups based on the morphology of the opisthohaptor, the caudal organ used for attaching to the host. With dactylogyrids, gyrodactylids fall into the common Monopisthocotylea type of monogeneans, which have a single large attachment organ with multiple large anchors or hooks (Figure 4). Together these 2 super families constitute the most economically important monogenean parasites of cultured fish and have been responsible for several rather notorious and costly declines in wild fish populations when inadvertently introduced into native stocks in Europe and the United States.[18] In Europe, the introduction was likely through transport of fish from one river to another for restocking; however, anglers moving between fishing sites without disinfecting their fishing gear may also be responsible for some of the geographic spread of gyrodactylids in the wild.[5]

Gyrodactylids and other monopisthocotyleans subsist on the superficial skin and gill layers of the host, causing irritation that can be inapparent or can manifest as clouding of the skin, focal reddening, epithelial hyperplasia, mucus overproduction, hemorrhage, and pruritus (indicated by flashing by affected fish). Ragged or damaged tails or fins may also be seen.[11,20,26] Deep skin wounds, secondary bacterial, fungal, or viral infections leading to mortality and energetic compromise due to increased flashing behavior are possible complications of gyrodactylid infection.[5,13,21] Even fish with low external parasite burdens can have elevated cortisol levels, causing immunosuppression that can render the host further susceptible to secondary infections.[5] In addition, severe emaciation of the damaged skin with heavy worm burdens likely disrupts the osmoregulatory capacity of the fish, adding another stressor.[5] Depending on the host species, there is considerable variation in the host response to the parasites, which is thought to dictate the severity of the infection to some degree; resistant salmon species exhibit limited proliferation of epithelial cells, therefore producing less organic matter for the parasites to subsist on and allowing time for the fish's immune system to attack the organisms before they propagate.[5] Domesticated *O. mykiss* is a species that appears to be particularly susceptible to some gyrodactylids. In fact, infections by various species, including *Gyrodactylus derjavini*, *G. salaris*, *G. colemanensis*, *G. salmonis*, *G. arcuatus*, *G. nerkae*, and *G. masu*,[1,6,8,16,19,23] have been documented in both captive and wild populations of rainbow trout, whereas other host fish seldom exhibit epidemic infections.[5]

Monogenean infections with low numbers of organisms may be subclinical, but in overcrowded, unsanitary, or suboptimal water quality conditions, viviparous monogeneans like gyrodactylids can reproduce rapidly and reach large numbers due to their direct life cycle. Transmission may occur by direct contact between fishes, contact between fish and detached worms on tank surfaces or equipment, and via worms floating in the water column.[5,13,21,22] Diagnosis is typically by microscopic examination of gill-clip or skin-scrape wet mounts, which often reveal parasites moving in a characteristic stretch-and-recoil motion while anchored in tissue. Identifying the species or even genus is not necessarily required for successful treatment, but when desired, live or preserved samples can be sent to a reference laboratory for specific taxonomic identification.[18]

The success of treatment can vary depending on the therapy chosen and the species or population of monogenean parasite. For captive fish, common treatment options include freshwater or saltwater baths (for marine or freshwater monogeneans, respectively) and prolonged immersion in formalin, organophosphates, mebendazole, or praziquantel (which may also be administered orally).[2,9,18,22,25,27,28] Aluminum sulfate, hydrogen peroxide, and benzocaine have also been documented as methods of treatment or control of some monogenean infections.[18] Rotenone has been used to completely eliminate infections among affected fish populations in the wild, including in 28 Norwegian rivers, which then were restocked with disease-free hatchery fish.[5] However, this option is obviously an extreme measure for captive fish and has not been proven to permanently eradicate the parasites in every case.[5] Formalin has long been used for the treatment of gyrodactylid infections but is currently a less desirable treatment due to its carcinogenic and mutagenic effects; formalin also can cause changes in gill structure and the epidermis. Organophosphates are often effective, but the parasites can develop resistance to these products, particularly with regular use.[18] For our facility, the choice of treatment was influenced heavily by the fact that no mechanism existed for containing effluent water and removing chemicals or medications prior to entering the watershed. Therefore, saltwater baths were deemed the best treatment option in this situation, providing good efficacy without adverse environmental consequences.

One limitation of saltwater baths as treatment for monogenean parasites is that the concentration required to effectively kill the parasites may not be tolerated by the fish, depending on the parasite and fish species involved. Freshwater monogeneans can rapidly become salt tolerant.[4,24] As a result, caution must be taken to ensure that the salinity change is great enough to kill the gyrodactylids but not the fish. Because gyrodactylids are viviparous, neutralization or elimination of eggs is not a concern, and treatment for the adult parasites is all that is required.[7,18] Rainbow trout can be anadromous (typically referred to as steelhead or steelhead trout) and therefore have a relatively high tolerance for saltwater compared with many other freshwater species.[20] They have been shown to have no change in blood pH or bicarbonate concentration after an abrupt change from freshwater to seawater.[3,7]

Treatment of small salmonids, as with most fish, requires caution when increasing salinity exposure. Fish that are less than 5 g in body weight generally should not be exposed to salt concentrations greater than 10 g/L, whereas those less than 100 g generally should not be exposed to greater than 20 g/L.[18] In contrast, some species of gyrodactylids can survive from 1 to 10 d at 10 g/L salinity and for nearly 2 d at 20 g/L.[5,24] Therefore, prior to instituting treatment in these rainbow trout, we performed a trial treatment of fish of different sizes to establish a relatively high but well-tolerated salt bath dose and duration; 20 g/L for 20 min was found to be acceptable for fish of all sizes in this population. Total cumulative mortality before treatment was 167; during treatment, cumulative mortality was reduced to 67, and after treatment, cumulative mortality was near 0.

Laboratory-based predator–prey interaction studies involving fish, like the one we describe here, often rely on fish hatcheries operated by either state or federal wildlife management agencies as sources for experimental animals. Such facilities are particularly useful for obtaining adequate numbers of smaller, prey-sized fish, given that these can be difficult to collect and transport effectively from the natural environment. Much effort is devoted to maintaining the health of fish produced in these facilities. Hatchery managers work directly with agency fish health professionals, pathologists, and veterinarians to ensure that fish raised and ultimately released into the environment are healthy. Consequently, hatchery-raised fish are typically naïve to some naturally occurring pathogens. Under laboratory conditions, if a population of animals is exposed to a new pathogen while subject to other sources of stress associated with experimental procedures, infection levels can quickly reach epizootic levels.

In the case we present, the most likely source of the parasitic organisms introduced into the population of hatchery-reared rainbow trout was the wild-captured cutthroat trout, which were transiently cohoused with the rainbow trout during acclimation in the unused holding tank and during pilot and experimental trials in the arena. Cutthroat trout were not physically evaluated for the presence of gyrodactylids by skin scrapes or gill biopsies, because this population was not exhibiting any morbidity or mortality. In addition, only a few cutthroat trout were available at the time of this event, and we opted to avoid handling these fish unnecessarily, to prevent their acclimation to humans and their exposure to additional stressful interactions that might have affected their use for this experimental study. Cutthroat trout are susceptible to infection by gyrodactylids and have found to be infected with gyrodactylids in the Pacific Northwest.[10,16] Several factors changed just before and during the experimental period that are important to note: 1) to reduce stress on the predators, researchers stopped placing cutthroat trout directly into the unused rainbow trout holding tank at 1 mo prior to running experiments; 2) this change coincided with a reduction in feeding frequency of the rainbow trout (from every other day to every 3 d) to help to maintain them at the desired body size and to achieve a specific behavior in the experimental arena; and 3) overall handling of predators and prey increased greatly during the experimental period.

The reduction in feeding, their increased handling, and pursuit by predators during the experimental period likely augmented stress levels in the rainbow trout, ultimately leading to the rapid increases in gyrodactylid infection levels and fish morbidity and mortality. The 2 most likely pathways of gyrodactylid exposure in this situation were direct exposure to cutthroat trout and fomite transmission via inadequately disinfected equipment used to transport fish between holding tanks and the experimental arena. Although monogeneans cannot survive longer than 2 wk off of a host, environmental control is still important, because chemical treatment baths alone may not eradicate all organisms.[18] Therefore, appropriate disinfection of nets and tanks with povidone–iodine between experimental groups was important in controlling the infection. In addition, alleviating crowding can help to reduce transmission; although not a desirable preventive measure for our colony, crowding nonetheless was reduced subsequent to the culling of fish that still had signs of illness after saltwater bath treatments.

Our rainbow trout may have been exposed to gyrodactylids or had a low level of infection on their arrival from the hatchery. In this case, the stress of shipment, new housing, husbandry, and experimental manipulations might have caused a rapid proliferation of parasites within this population. Because we did not evaluate this population of rainbow trout on their arrival at our facility, we do not know whether a baseline gyrodactylid infection existed. However, additional clinical cases of gyrodactylid were not identified within this population of rainbow trout after the completion of the saltwater bath treatments and implementation of husbandry modifications. Subsequent populations (n = 2; 1620 to 2115 fish each) of rainbow trout obtained from the same hatchery and transferred to BBC, did not develop clinical infections of gyrodactylids, nor did populations of coho salmon housed on site and used for various experimental procedures. Due to a shift in the research focus,[12] direct exposure to cutthroat trout within the holding tanks of these new populations of rainbow trout was not performed. In addition, new fish were never returned to the stock tanks after being exposed to cutthroat trout in the experimental arena; and the subsequent populations of rainbow trout were housed on site longer and endured higher levels of experimental usage than did the original infected population, further supporting the notion that cutthroat trout were an important pathway for transmission. Finally, given that the fresh water well at BBC is 300 feet below the surface, it is unlikely that live-bearing gyrodactylids were introduced into this fish population via the water source.

Research endeavors using rainbow trout are diverse. For example, rainbow trout and embryos have been used to detect the presence of carcinogens and have demonstrated the carcinogenicity of 28 compounds, most of which are hepatotoxic, including aflatoxin B_1 and its metabolites.[4,14] Rainbow trout make an excellent cancer model due to their low rates of spontaneous neoplasms, sensitivity to mammalian carcinogens, and short latency periods.[4] More recently, studies using cultured ovarian follicles from rainbow trout have explored the involvement of apoptosis in teleost ovarian development and the regulatory importance of certain hormones and growth factors, some of which play roles in mammalian and avian ovaries, on this apoptosis.[15] Because rainbow trout continue to be used for research purposes and to be farmed for human consumption, successful, environmentally friendly management of sporadic diseases such as parasitic infections are increasingly important. This case study highlights the inherent risks of fish experiments involving the mixture of untreated, nonquarantined fish from wild-caught and hatchery sources and the successful treatment of gyrodactylid infections in *Oncorhynchus mykiss* by using saltwater baths and intensified disinfection procedures of equipment with povidone–iodine.

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